IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of:

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D. Gassaway
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Andreas L.J. De Meere et al.

Serial Number: 07/672,509 Group Art Unit: 424

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For: STABILIZED GONADOTROPIN CONTAINING PREPARATIONS

CLAIM TO PRIORITY UNDER 35 USC 119

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

April 16, 1988

Sir:

The benefit of the filing date of the following prior foreign application is hereby requested for the above-identified application, and the priority provided in 35 USC 119 is hereby claimed:

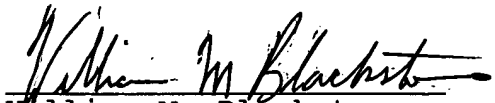
European patent application 90200665.9 dated March 20, 1990

In support of this claim, the requisite certified copy of said original foreign application is filed herewith.

It is requested that the file of this application be marked to indicate that the Applicant has complied with the requirements of 35 USC 119 and that the Patent and Trademark Office kindly acknowledge receipt of this document.

In the event any fees are required with this paper, please charge our Deposit Account No. 02-2334, for which purpose duplicate copies are enclosed.

Respectfully submitted,



William M. Blackstone
Attorney for Applicants
Registration No. 29,772

ORGANON TEKNIKA CORPORATION
BIOTECHNOLOGY RESEARCH INSTITUTE
1330-A Piccard Drive
Rockville, Maryland 20850-4373
Tel: (301) 258-5200

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Bescheinigung

Die angehefteten Unterlagen stimmen mit der ursprünglich eingereichten Fassung der auf dem nächsten Blatt bezeichneten europäischen Patentanmeldung überein.

Certificate

The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Attestation

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Den Haag, den
The Hague,
La Haye, le 20. 03. 91

Der Präsident des Europäischen Patentamts
Im Auftrag
For the President of the European Patent Office
Le Président de l'Office européen des brevets
p. o.

M.B. RIJLING

Patentanmeldung Nr.
Patent application no. 90200665.9
Demande de brevet n°

Blatt 2 der Bescheinigung
Sheet 2 of the certificate
Page 2 de l'attestation

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Demande n°:

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Bezeichnung der Erfindung:
Title of the invention: Stabilized protein preparations.
Titre de l'invention:

In Anspruch genommene Priorität(en)
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STABILIZED PROTEIN PREPARATIONS

Background of the Invention

Field. This invention relates to pharmaceutical compositions generally, and to stabilized protein preparations specifically.

State of the Art: Relatively pure protein preparations are commercially available. For example, compositions containing naturally derived human menopausal gonadotropin ("HMG") and naturally derived human chorionic gonadotropin ("HCG") are available as freeze-dried preparations under the trade designations "Humegon" and "Pregnyl," respectively, from Organon International, bv of Oss, NL. Pregnant mare gonadotropin is also available in a freeze dried form from the same company.

A bulking agent, e.g. mannitol, is added to these preparations before lyophilization. They do not require the addition of a stabilizer to ensure an adequate shelf-life. Evidently whatever natural contaminants remain after the purification process act to stabilize the preparations in freeze-dried form.

Recently however, with the advent of more effective production and purification techniques, preparations of certain very pure proteins are insufficiently stable. They degrade in a relatively short time, losing activity. In order to prevent or slow down this degradation, attempts were made to freeze-dry (lyophilize) the preparations. Lyophilization has only been partially successful however.

For example, in U.S. Patent No. 4,806,524 to Kawaguchi et al it is stated that a freeze-dried

preparation of erythropoietin ("EPO") containing no added stabilizer degrades to 60% of its original activity (as determined by the fasted rat method of Goldwasser et al, Methods in Enzymol., vol. 37, pp. 109-121 (1975)) in one month at 37^{o} centigrade (C).

Kawaguchi et al goes on to disclose various agents which assertedly act to stabilize EPO preparations. Included among these agents are disaccharides including sucrose; polysaccharides such as dextran; organic salts including sodium and potassium citrate; inorganic salts including sodium dihydrogen phosphate and disodium hydrogen phosphate; and other compounds such as polyethylene glycol 4000. Kawaguchi et al goes on to state that two or more of these stabilizers may be used if their total amount is within the range of 0.1 to 10,000 parts by weight per part by weight of EPO.

Example 9 of Kawaguchi et al discloses an aqueous solution containing 1 part, by weight, EPO; 50 parts dextran 40; 98 parts $NaH_2PO_4 \cdot 2H_2O$; 12 parts citric acid monohydrate; 500 parts sucrose; and 10^5 parts water. A potential problem with such a preparation is that compounds, such as dextran 40, have the well-known potential of causing allergic reactions, such as anaphylaxis, in the patient. Furthermore, dextran 40 is contraindicated in the case of renal failure, which is associated with the main indication for EPO.

A need exists for a relatively non-allergenic lyophilisate containing protein, such as EPO, which is stable over a sufficiently long period of time for the product to be manufactured, shipped, and stored prior to use.

Summary of the Invention

Generally, the invention includes a lyophilized protein preparation which contains a dicarboxylic acid salt stabilizer. "Dicarboxylic acid," as used herein, means

an organic acid having two or more carboxylic acid moieties (e.g HOOC-R-COOH). The protein will generally be a simple protein, a complex protein, such as a glycoprotein, or a mixture thereof. The particular protein or proteins will be in admixture with, and at least partially capable of stabilization by, the particular stabilizer in lyophilized systems. The preparation will contain a sufficient amount of dicarboxylic acid salt to stabilize the protein in its freeze-dried form for a desired time at a desired temperature.

Typical dicarboxylic acid salts are salts of citric acid, tartaric acid, aspartic acid, or mixtures of these acids. The protein will generally be erythropoietin or one or more of the gonadotropin derivatives (as used herein "gonadotropins"), such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), human chorionic gonadotropin (HCG), or luteinizing hormone (LH). The preparation can further include a non-reducing disaccharide, such as sucrose or trehalose.

The invention also includes a method of stabilizing an essentially pure protein, such as erythropoietin, ß-glucuronidase, or one of the gonadotropins, in lyophilized form, which method involves mixing the protein, in solution, with a sufficient amount of a dicarboxylic acid salt to stabilize the protein in the lyophilized form, and then freeze-drying the resulting solution to form a stabilized lyophilisate of the protein.

The invention further includes the reconstituted injectable preparation made from the lyophilisate. The injectable preparation consists essentially of aqueous solution of water for injection, the protein, a non-reducing sugar, an anti-adsorption agent, and the dicarboxylic acid salt.

Brief Description of the Drawings

FIG. 1 is a HPSEC (high performance size exclusion chromatography) profile of lyophilized recombinant DNA produced FSH ("rFSH") stabilized with sodium citrate.

FIG. 2 is another HPSEC profile showing oligomer formation of the rFSH when no sodium citrate is present.

FIG. 3 is a graph depicting the correlation between ionic strength (X 1000) versus the amount of recovery of recombinant FSH activity after 1 month at 60° C using various stabilizers.

Description of the Preferred Embodiments

A. Proteins-

Preferred proteins include FSH, TSH, HCG, LH, EPO, derivatives and mixtures thereof, with or without other protein components.

Follicle stimulating hormone, thyroid stimulating hormone, human chorionic gonadotropin, and luteinizing hormone are all chemically related proteins which consist of α and ß subunits. The α subunits of these proteins are identical or nearly so.

Follicle stimulating hormone is a hormonal glycoprotein of the anterior pituitary required for normal reproductive function. Follicle stimulating hormone has been used to stimulate development of ovarian follicles for in vitro fertilization, and has also been used clinically to stimulate follicular maturation in anovulatroy women with chronic anovulatory syndrome or luteal phase deficiency. Follicle stimulating hormone may be at least partially isolated from natural sources, such as human urine. Recombinant follicle stimulating hormone and/or LH may be prepared as described in Keene et al "Expression of Biologically Active Human Follitropin in Chinese Hamster Ovary Cells," The Journal of Biological Chemistry, Vol. 264, pp. 4769-4775 (25 March 1989), the contents of which

are incorporated by this reference. As used herein, follicle stimulating hormone (FSH) includes the compound's analogs, and its recombinant, natural, deglycosylated, unglycosylated, modified glycosylated, and other forms.

Erythropoietin is also known as "epoetin alfa," and is a glycoprotein which is used in the treatment or diagnosis of anemia, especially in the treatment of anemia in patients with end-stage renal disease. It has also been used in the treatment of rheumatoid arthritis. Recombinant erythropoietin (rEPO) is available under the trade designation Epogen from Amgen Corporation of Thousand Oaks, California, U.S.A. Erythropoietin is also available, reputedly under the trade designation Epogin, from Chugai Pharmaceuticals of Japan. For information concerning the production and purification of EPO, reference may be had to European Patent Applications 148,605 (corresponding to U.S. Patent No. 4,703,008) and 209,539 (corresponding to U.S. Patent No. 4,677,195) respectively. As used herein, erythropoietin includes the compound's analogs and the recombinant, natural, deglycosylated, unglycosylated, modified glycosylated (e.g. European Patent Application 267,678), and other forms of the glycoprotein.

ß-glucuronidase is a protein which currently has utility as a research reagent. It is commercially available from Sigma Chemical Company of St. Louis, MO, U.S.A.

Especially preferred proteins for use with the stabilizer sodium citrate are rEPO and rFSH, due to these compounds' ability to be stabilized with the stabilizers.

The most preferred protein is rFSH. Follicle stimulating hormone purified from natural sources is generally only partially purified. The impurities seem to act to stabilize the FSH somewhat. With rFSH, however the impurities are not present, and thus the FSH is more susceptible to rapid degradation and freeze-drying losses.

Any protein used is preferably present in the lyophilisate preparations in a quantity sufficient to form a therapeutically useful concentration of the protein after dilution, generally for parenteral (e.g. subcutaneous or intravenous) administration, with a specified amount of an aqueous solution (e.g. distilled water for injection or sterile normal saline) to form a volume of preparation contemplated for use. As used herein, an aqueous solution is a solution containing water as a primary, but not necessarily the only, solvent. For example, a container containing an EPO lyophilisate may contain from 250 to 15,000 units of EPO, more typically 2000 to 10,000 units. A container containing FSH may contain 1 to 1000 micrograms (μg) of FSH (e.g. 75 international units is considered a therapeutic amount). Preferably, the highest reasonable amount of protein possible will be present in a container, since the greater the amount of protein present, generally the more stable the preparation.

Useful doses for the proteins are known to medical practitioners. For example, one (1) ml of a parenteral solution containing 3500 units of EPO administered intravenously on a thrice weekly basis is generally suitable for the initial therapy of a 70 kilogram (154 lbs.) man suffering from anemia induced by chronic renal failure. In another instance, 15 ml of a 500 U / ml solution of EPO may be administered on a twice weekly basis to treat anemia.

B. Stabilizers-

As used herein, "stabilize" is a relative term. To stabilize with a stabilizing agent or compound means the ability to prevent or delay a decrease in the protein's activity with the stabilizing agent. For example, a preparation would be deemed "stabilized" if, with the addition of a stabilizing compound ("stabilizer"), it took longer (e.g. 2 weeks instead of 1 week) to degrade at a set temperature, thus losing some of its <u>in vivo</u> or <u>in</u>

vitro activity in comparison to the preparation sans the stabilizer.

A protein's activity may be determined by known methods relating to the particular protein. One measure of activity can be made by measuring the amount of (inactive) oligomers formed over time. Oligomer formation in a sample can be determined by HPSEC.

Other methods of determining the residual activity of, for example, rFSH or rEPO include enzyme immunoactivity assay ("EIA") as described in U.S. Patent Reissue No. 32,696 to Schuurs et al; the previously referenced fasted rat method of Goldwasser et al for EPO; a kit available under the trade designation "Biomerieux" of Macy 'Letoil, FR for FSH; and in vitro bioassay of both FSH and LH as described in Mannaerts et al, "Applications of in vitro Bioassays for Gonadotropins," Neuro-endocrinology of Reproduction, pp. 49 - 58 (Elsevier Science Publishers bv, Amsterdam, NL 1987).

Preferred stabilizers for use with the preparations are salts of dicarboxylic acids such as citric acid, tartaric acid, aspartic acid, and mixtures thereof. Preferred salts are the sodium, potassium, lithium and ammonium salts of such dicarboxylic acids. Sodium citrate is especially preferred if the preparation is ultimately to be used to treat a patient suffering from renal failure. Another dicarboxylic acid salt is sodium glutamate. The presence of a dicarboxylic acid salt stabilizer acts to stabilize the enzymatic mixtures, especially at relatively higher temperatures over longer periods of time.

Concentrations of dicarboxylic acid salt stabilizers sufficient to form a solution having an ionic strength of greater than 0.050 are preferred in EPO and FSH compositions containing between 0.1 and 1000 µg of EPO or FSH. Especially preferred are those solutions having an ionic strength of between 0.250 and 0.350 which will generally stabilize, for example, rFSH stored at one month at 60°C to yield a 75% recovery of rFSH. Calculation of ionic

strength is well-known to those skilled in the art, for example see Chase, et al, Remington's Pharmaceutical Sciences, pp. 223-224, 228, and 233 (16th ed. 1980, Mack Publ. Co. of Easton, PA, U.S.A.), the contents of which are incorporated by this reference.

Concentrations of 2.5 to 17.5 milligrams per milliliter (mg/ml) of sodium citrate in solution are generally sufficient to stabilize lyophilized rFSH in the amounts described herein.

C. Non-reducing Sugars-

The compositions to be freeze-dried preferably contain a non-reducing sugar such as sucrose or trehalose. The incorporation of such a sugar, e.g. sucrose, acts to increase the "collapse (or 'shrinkage') temperature" at which the lyophilization of the solution takes place. This increase in temperature simplifies the entire freeze-drying process. An especially preferred non-reducing sugar for this use is sucrose in its amorphic state.

The amount of non-reducing sugar present in the solution to be lyophilized will generally be dependent upon the amount of dicarboxylic acid salt stabilizer present. For example, the weight ratio of non-reducing sugar to dicarboxylic acid salt will generally vary between 50 : 1 to 10 : 3, with a preferred concentration being about 3.3 : 1 in the case of sucrose to sodium citrate. Especially preferred is a solution containing 50 mg/ml sucrose and 14.7 mg/ml sodium citrate which also yields an optimal lyophilisate in terms of physical characteristics.

In the presently most preferred embodiments, the amount of sucrose will be sufficient to raise the collapse temperature from -38^{o} C to about -25^{o}C as determined by differential scanning calorimetry. The resulting lyophilisate "cake" remains amorphous and stable for relatively longer periods of time.

D. Anti-absorption agents-

Anti-adsorption agents are preferably added to the lyophilized composition to prevent adsorbance of the protein to the walls of the container in which the compositions are contained, thus preventing a possible decrease in concentration. Certain anti-adsorption agents (e.g. polysorbates) also act as "cryoprotectants" protecting the protein during the lyophilization process.

Preferred anti-adsorption agents are nonionic surfactants such as Polysorbate 20, NF (Tween 20 available from Atlas Chemical Company), Polysorbate 80, NF (Tween 80 available from Atlas Chemical Company), Brij 35 (available from ICI Pharmaceuticals of Great Britain), and Pluronic F123 (available from BASF of Ludwigshafen, W. Germany). Polysorbate 20, NF is especially preferred.

Polysorbate is preferably understood as meaning a polysorbate which meets the specification of USP/NF XXII, which is published as "The National Formulary", p. 1763 and 1967, Official from 1 Jan. 1990 (22nd ed., U.S. Pharmacopeial Convention, Inc. 1989), the contents of which are incorporated by this reference.

An anti-adsorption agent or anti-adsorption agents will be present in such amounts that adsorption of the protein onto container walls, or walls of vessels during processing, is decreased. Illustratively, amounts of Polysorbate 20 sufficient to form a concentration between 0.1 and 0.2 mg / ml in the ultimate solution for use are preferred. Concentrations higher than this tend to lead to oligomer formation, and thus decreased activity.

E. Pharmaceutical Compositions-

The stable lyophilized preparation of the instant invention can be prepared by admixing the selected protein in aqueous solution with a sufficient amount of a dicarboxylic acid salt stabilizer to stabilize the protein, and a sufficient amount of a non-reducing sugar to increase the collapse temperature from -38°C to greater than -25°C. Temperatures greater than -35°C are pre-

ferred. Optionally, the selected anti-adsorption agent may also be added. The solution is then filtered, placed into containers and then freeze-dried to form a stabilized lyophilisate. Freeze-drying techniques are well-known to those of skill in the art. For more information, reference may be made to several texts, including Goldblith et al, Freeze Drying and Advanced Food Technology, (Academic Press, Inc., London, GB 1975). Preferred residual water content in the lyophilisate cakes are between 1 and 5 %. Aseptic techniques should be used throughout the procedure. Freeze-driers are available from manufacturers such as Leybold or Edwards. Using such a procedure, or modifications thereof, several different compositions may be prepared.

An especially preferred composition contains rFSH in admixture with a stabilizer which is a salt of a dicarboxylic acid, wherein the dicarboxylic acid is selected from the group consisting of citric acid, tartaric acid, aspartic acid, and mixtures of these acids.

Another preferred lyophilized preparation contains, in admixture, a dicarboxylic acid salt stabilizer, a protein capable of stabilization by the amount of stabilizer present in the preparation, and trehalose. This preparation further include sodium biphosphate in admixture with the stabilizer, protein, and non-reducing sugar. Especially preferred salts for such preparations are sodium aspartate, sodium citrate, and sodium tartrate.

Another preferred stable lyophilized preparation contains, in admixture, a stabilizer such as a salt of tartaric or aspartic acid, a protein capable of stabilization by the amount of stabilizer present in the preparation, and a non-reducing sugar. The preparation may further include disodium biphosphate in admixture with the stabilizer, protein, and non-reducing sugar. Especially preferred non-reducing sugars are trehalose and sucrose. An especially preferred stabilizer in such preparations is sodium aspartate.

Another highly preferred stabilized lyophilisate consists essentially of a protein; a sufficient amount of a dicarboxylic acid salt stabilizer to stabilize the protein in freeze dried form; a disaccharidic non-reducing sugar; an anti-adsorption agent to prevent said protein from adsorbing onto a container containing the lyophilisate; and less than five percent residual water. In such a lyophilisate the protein will be FSH or EPO; the dicarboxylic acid salt stabilizer will be selected from the group consisting of salts of citric acid, tartaric acid, and aspartic acid; the disaccharidic non-reducing sugar will either be sucrose or trehalose, and the anti-adsorption agent will be selected from the group consisting of Tween 20, Tween 80, Brij, or pluronic acid. This lyophilisate is especially preferred since, among other things, it has been discovered that the addition of further "stabilizers," such as mannitol, maltose, or either of them actually act to destabilize the lyophilisate in terms of activity (see, e.g. EXAMPLEs I and III).

Methods for making parenteral preparations and intravenous admixtures are disclosed in Remington's Pharmaceutical Sciences, pp. 1463-1497, the contents of which are incorporated by this reference. However, caution must be exercised since although the stabilized compositions are compatible with infusion liquids, the infusion liquid used preferably should not contain reducing sugars. The preferred pH of the resulting solution for use should be between 6 and 8, especially 7.

The invention is further explained by reference to the following EXAMPLES:

EXAMPLE I

A. Stabilization of rFSH utilizing various disaccharides.

Aqueous solutions containing 150 units of rFSH were prepared. The solutions were divided into three groups and each group was mixed with (1) 50 mg/ml maltose / 14.7

mg/ml sodium citrate; (2) 50 mg/ml trehalose / 14.7 mg/ml sodium citrate and (3) 50 mg/ml sucrose / 14.7 mg/ml sodium citrate. All three solutions also contained 0.2 mg/ml Polsorbate 20, NF. The three groups of solutions were freeze-dried, and the resulting lyophilisate allowed to sit for four weeks at 60°C. The lyophilisates were then tested for activity (as determined by EIA) with the following results:

Compound:	(1)	(2)	(3)
Percentage activity:	40%	89%	87%

This EXAMPLE shows that non-reducing disaccharides aid stability better than reducing disaccharides.

B. Stabilization of rFSH with Sodium Citrate

Two lyophilized samples are made. The first sample contains 75 Units rFSH, 25 mg amorphic sucrose, 7.35 mg sodium citrate, and 0.1 mg Polysorbate (Tween) 20. The second sample contains 75 Units rFSH, 25 mg amorphic sucrose, and 0.2 mg Tween 20. The pH of both samples was adjusted to 7. The first sample is stored for 3 months at 50° C, reconstituted with purified water, and analyzed by HPSEC. The resulting profile is shown in FIG. 1. The second sample, not containing sodium citrate, is stored for 6 months at 50° C, reconstituted with purified water, and analyzed by HPSEC. The resulting profile is shown in FIG. 2.

The profile of the first sample shows no degradation products while the profile of the second sample shows almost exclusively oligomeric products.

EXAMPLE II

Stabilization of rEPO.

Solutions for lyophilization containing approximately 5000 units of rEPO per vial were studied for the effects of the addition of various compounds on stability. The rEPO samples also contained 2 mg of man-

nitol per vial. In Sample 1, 25 mg of sucrose was added. In Sample 2, 25 mg of sucrose and 7.5 mg of sodium citrate were added. In Sample 3, 25 mg of sucrose and 0.1 mg of Polysorbate 20 (Tween 20 of Atlas Chemical Company) were added. In Sample 4, 25 mg of sucrose, 7.5 mg of sodium citrate and 0.1 mg of Polysorbate 20 were added. The residual amount of activity (determined by EIA), given as a percentage of original activity after reconstitution with purified water, is given in TABLE A.

TABLE A

Sample No.	4 weeks @ 4°C	4 weeks @ 60° C	8 weeks @ 30° C	8 weeks @ 50° C	8 weeks @ 60° C
1	90	52	58	29	17
2*	75	78	59	45	33
3	88	< 7	37	17	9
4*	92	71	43	40	31

* denotes the presence of citrate.

As can be seen from these results, the presence of the dicarboxylic acid salt, sodium citrate, acted to stabilize the rEPO mixtures especially at higher temperatures over longer periods of time.

EXAMPLE III

Stabilization of ß-glucuronidase

Solutions for lyophilization containing 250 units of the relatively large (270K daltons) protein ß-glucuronidase (Sigma Chemical Company) were studied for the effects of the addition of various compounds on stability. The amount of protein was varied between 0.025 and 0.30 mg/ml, sodium citrate was varied from 0 to 2 molar, and the sugar, sucrose or maltose, were varied from 0 to 10 % (weight/volume). Activity of ß-glucuronidase was determined by the method of Fishman (Nord, F. F. (ed.), Advances in Enzymology, (Int. Publishers Ltd., New York, 1988), wherein one unit of activity was defined as the

release of 1 μg phenolphthalein from phenolphthalein-glucuronic acid in one hour at 56° C.

The results showed that the ratio of citrate to protein was the most important factor affecting stability in solution and in the resulting freeze dried cakes. Optimal results were obtained with a ratio of 200 to 400 (weight/weight), wherein 80 to 90 % activity was recovered. The presence of the non-reducing sugar, sucrose, aided stability better than the presence of the reducing-sugar, maltose.

EXAMPLE IV

Effects of other compounds on stabilization

To determine the effect of other compounds on stability, other samples were made. Sample 2 and Sample 4 were as described in EXAMPLE II. Samples 1A and 3A were identical to Samples 2 and 4 respectively except that they each contained 7 mg of mannitol instead of the 2 mg of Samples 2 and 4. Sample 5 contained 5000 Units EPO, 2 mg mannitol, 7.5 mg citrate 0.1 mg Polysorbate 20, NF, and 25 mg of the reducing disaccharide maltose instead of sucrose. The results of these tests are given in TABLE B.

TABLE B

Sample No.	4 weeks @ 4° C	4 weeks @ 60° C	8 weeks @ 30° C	8 weeks @ 50° C	8 weeks @ 60° C
1A	100	NA	43	33	19
2	75	78	59	45	33
3A	90	<22	40	24	18
4	92	71	43	40	31
5	75	< 4	38	21	≤ 2

These results demonstrate that the presence of other "stabilizers" tends to actually destabilize the protein in lyophilisate form.

EXAMPLES V-VIII

Other dicarboxylic acid salt stabilizers.

As depicted in FIG. 3, samples containing rFSH and various stabilizers were made, lyophilized, and tested for activity after 1 month storage at 60° C. FIG. 3 depicts the correlation between ionic strength (X 1000) of the particular stabilizer versus the percentage recovery (as determined by EIA).

The stabilizers tested were sodium citrate, both separately (V) and with 3.0 to 9.1 mg Na_2HPO_4 per ampule (VI); sodium tartrate (VII); and sodium aspartate (VIII). Concentrations were determined in terms of ionic strength as shown in FIG. 3.

EXAMPLE IX

Attempted stabilization with Dextran 40.

Effects of the glucose polymer Dextran 40 for stabilization were examined. Sample 2 was as before. Sample 6 contained 5000 Units EPO, 2 mg mannitol, 12 mg Dextran 40 instead of sodium citrate, and 18 mg of sucrose. The results are given in TABLE C.

TABLE C

Sample No.	4 weeks @ 4° C	4 weeks @ 60° C	8 weeks @ 30° C	8 weeks @ 50° C	8 weeks @ 60° C
2	75	78	59	45	33
6	95	20	43	37	8

EXAMPLES X -XIV

Attempts were made to use mannitol (5 and 12.5 mg / vial), mannitol and Polysorbate 20, mannitol and glucose, and mannitol and hydrolyzed gelatin as stabilizers of rEPO. The lyophilisates using these compounds as stabilizers showed more degradation of the rEPO than those of the invention.

Claims

What is claimed is:

1. A stabilized protein lyophilisate comprising:

a stabilizer comprising a salt of a dicarboxylic acid, said stabilizer being in admixture with a protein capable of stabilization by the amount of stabilizer present in the lyophilisate.

2. A stabilized protein lyophilisate comprising, in admixture, a stabilizer consisting essentially of a salt of a dicarboxylic acid, a protein capable of stabilization by the amount of stabilizer present in the lyophilisate, and the non-reducing sugar trehalose.

3. A stabilized protein lyophilisate consisting essentially of a protein; a sufficient amount of a stabilizer, said stabilizer comprising the salt of a dicarboxylic acid, to stabilize said protein in freeze dried form a disaccharidic non-reducing sugar, and a non-ionic surfactant.

4. The stable lyophilisate of claim 1, claim 2 or claim 3, wherein said protein is selected from the group consisting of erythropoietin, luteinizing hormone, human chorionic gonadotropin, thyroid stimulating hormone, ß-glucuronidase, or follicle stimulating hormone.

5. A stabilized follicle stimulating hormone lyophilisate comprising recombinant follicle stimulating hormone in admixture with a stabilizer comprising a salt of a dicarboxylic acid, said salt being present in sufficient quantities to stabilize said recombinant follicle stimulating hormone.

6. A stabilized protein lyophilisate comprising, in admixture, a salt of tartaric acid or aspartic acid, a protein capable of stabilization by the amount of salt present in the lyophilisate, and a non-reducing sugar.

7. The stabilized protein lyophilisate of claim 2 or claim 6 further comprising disodium biphosphate in admixture with said stabilizer, protein, and non-reducing sugar.

8. The stabilized lyophilisate of claim 1, claim 2, claim 3, claim 5, or claim 7 wherein said dicarboxylic acid is selected from the group consisting of citric acid, tartaric acid, aspartic acid, and mixtures thereof.

9. The stable lyophilisate of claim 8 further including a non-reducing sugar in an amount three to fifty times by weight of the dicarboxylic acid salt.

10. The stable lyophilisate of any of claim 1, claim 2, claim 3, claim 4, claim 8, or claim 9 further including a second protein capable of stabilization by said dicarboxylic acid salt stabilizer.

11. The preparation of claim 6 wherein said salt is sodium aspartate.

12. The lyophilisate of claim 3 wherein the disaccharidic non-reducing sugar is present in amounts of three times, by weight, that of the dicarboxylic acid salt stabilizer.

13. A method of making a stable lyophilisate containing a protein selected from the group consisting of erythropoietin, luteinizing hormone, human chorionic gonadotropin, thyroid stimulating hormone, ß-glucuronidase, and follicle stimulating hormone, comprising:

admixing the selected protein in an aqueous solution with a sufficient amount of a dicarboxylic acid salt stabilizer to stabilize the selected protein in lyophilized form,

dissolving sucrose into said admixture in an amount three to fifty times, by weight, that of the dicarboxylic acid salt stabilizer, and then

freeze-drying the admixture at temperatures above -35^{o} centigrade, and preferably above -28^{o} centigrade, to form a stabilized lyophilisate.

14. An injectable admixture consisting essentially of water, one or more proteins, non-reducing sugar, anti-adsorption agent, and a dicarboxylic acid salt.

15. A method of making a stabilized protein lyophilisate comprising:

admixing, in an aqueous solution, at least one protein with an amount of dicarboxylic acid salt to adjust the ionic strength of said solution to between 0.050 and 0.350,

dissolving a non-reducing sugar in said admixture in an amount of about three to about ten times the amount, by weight, of the dicarboxylic acid salt, and

freeze-drying said admixture at a temperature greater than -29^{o} centigrade to form said stabilized protein lyophilisate.

Abstract of the Disclosure

Disclosed are lyophilized protein preparations containing a dicarboxylic acid salt stabilizer. The particular proteins (e.g. EPO, LH, TSH, FSH, HCG, or ß-glucuronidase) are in admixture with, and at least partially capable of stabilization by, the particular stabilizer in lyophilized form. The preparations contain a sufficient amount of dicarboxylic acid salt to stabilize the protein in its freeze-dried form for a desired time at a desired temperature. Typical dicarboxylic acid salts disclosed are the salts of citric, tartaric, and aspartic acids. The preparations preferably include a non-reducing disaccharide to increase the collapse temperature of the solution to be lyophilized. Methods of making the preparations in lyophilized form and the resulting injectable preparations are also disclosed.